

*Culture Procedures*, Texas Ag. Exper. Station Bulletin No. 1555 (1988), both of which are incorporated by reference in their entirety).

**[0097]** Recombinant protein expression in insect cells is achieved by viral infection or stable transformation. For viral infection, the desired gene is cloned into baculovirus at the site of the wild-type polyhedrin gene (Webb and Summers, *Technique* 2:173 (1990); Bishop and Posse, *Adv. Gene Technol.* 1:55 (1990); both of which are incorporated by reference in their entirety). The polyhedrin gene is a component of a protein coat in occlusions which encapsulate virus particles. Deletion or insertion in the polyhedrin gene results the failure to form occlusion bodies. Occlusion negative viruses are morphologically different from occlusion positive viruses and enable one skilled in the art to identify and purify recombinant viruses.

**[0098]** The vectors of present invention preferably contain one or more selectable markers which permit easy selection of transformed cells. A selectable marker is a gene the product of which provides, for example biocide or viral resistance, resistance to heavy metals, prototrophy to auxotrophs, and the like. Selection may be accomplished by co-transformation, e.g., as described in WO 91/17243, a nucleotide sequence of the present invention may be operably linked to a suitable promoter sequence. The promoter sequence is a nucleotide sequence which is recognized by the insect host cell for expression of the nucleotide sequence. The promoter sequence contains transcription and translation control sequences which mediate the expression of the protein or fragment thereof. The promoter may be any nucleotide sequence which shows transcriptional activity in the insect host cell of choice and may be obtained from genes encoding polypeptides either homologous or heterologous to the host cell.

**[0099]** For example, a nucleic acid molecule encoding a *D. v. virgifer* protein homologue or fragment thereof may also be operably linked to a suitable leader sequence. A leader sequence is a nontranslated region of a mRNA which is important for translation by the insect host. The leader sequence is operably linked to the 5' terminus of the nucleotide sequence encoding the protein or fragment thereof. The leader sequence may be native to the nucleotide sequence encoding the protein or fragment thereof or may be obtained from foreign sources. Any leader sequence which is functional in the insect host cell of choice may be used in the present invention.

**[0100]** A polyadenylation sequence may also be operably linked to the 3' terminus of the nucleotide sequence of the present invention. The polyadenylation sequence is a sequence which when transcribed is recognized by the insect host to add polyadenosine residues to transcribed mRNA. The polyadenylation sequence may be native to the nucleotide sequence encoding the protein or fragment thereof or may be obtained from foreign sources. Any polyadenylation sequence which is functional in the fungal host of choice may be used in the present invention.

**[0101]** To avoid the necessity of disrupting the cell to obtain the protein or fragment thereof, and to minimize the amount of possible degradation of the expressed polypeptide within the cell, it is preferred that expression of the polypeptide gene gives rise to a product secreted outside the cell. To this end, the protein or fragment thereof of the present invention may be linked to a signal peptide linked to the amino terminus of the protein or fragment thereof. A signal peptide is an amino

acid sequence which permits the secretion of the protein or fragment thereof from the insect host into the culture medium. The signal peptide may be native to the protein or fragment thereof of the invention or may be obtained from foreign sources. The 5' end of the coding sequence of the nucleotide sequence of the present invention may inherently contain a signal peptide coding region naturally linked in translation reading frame with the segment of the coding region which encodes the secreted protein or fragment thereof.

**[0102]** At present, a mode of achieving secretion of a foreign gene product in insect cells is by way of the foreign gene's native signal peptide. Because the foreign genes are usually from non-insect organisms, their signal sequences may be poorly recognized by insect cells, and hence, levels of expression may be suboptimal. However, the efficiency of expression of foreign gene products seems to depend primarily on the characteristics of the foreign protein. On average, nuclear localized or non-structural proteins are most highly expressed, secreted proteins are intermediate, and integral membrane proteins are the least expressed. One factor generally affecting the efficiency of the production of foreign gene products in a heterologous host system is the presence of native signal sequences (also termed presequences, targeting signals, or leader sequences) associated with the foreign gene. The signal sequence is generally coded by a DNA sequence immediately following (5' to 3') the translation start site of the desired foreign gene.

**[0103]** The expression dependence on the type of signal sequence associated with a gene product can be represented by the following example: If a foreign gene is inserted at a site downstream from the translational start site of the baculovirus polyhedrin gene so as to produce a fusion protein (containing the N-terminus of the polyhedrin structural gene), the fused gene is highly expressed. But less expression is achieved when a foreign gene is inserted in a baculovirus expression vector immediately following the transcriptional start site and totally replacing the polyhedrin structural gene.

**[0104]** Insertions into the region -50 to -1 significantly alter (reduce) steady state transcription which, in turn, reduces translation of the foreign gene product. Use of the pVL941 vector optimizes transcription of foreign genes to the level of the polyhedrin gene transcription. Even though the transcription of a foreign gene may be optimal, optimal translation may vary because of several factors involving processing: signal peptide recognition, mRNA and ribosome binding, glycosylation, disulfide bond formation, sugar processing, oligomerization, for example.

**[0105]** The properties of the insect signal peptide are expected to be more optimal for the efficiency of the translation process in insect cells than those from vertebrate proteins. This phenomenon can generally be explained by the fact that proteins secreted from cells are synthesized as precursor molecules containing hydrophobic N-terminal signal peptides. The signal peptides direct transport of the select protein to its target membrane and are then cleaved by a peptidase on the membrane, such as the endoplasmic reticulum, when the protein passes through it.

**[0106]** Another exemplary insect signal sequence is the sequence encoding for *Drosophila* cuticle proteins such as CP1, CP2, CP3 or CP4 (Summers, U.S. Pat. No. 5,278,050; herein incorporated by reference in its entirety). Most of the 9 kb region of the *Drosophila* genome contains genes for the cuticle proteins has been sequenced. Four of the five cuticle